Supplement 1. Immunofluorescence study shows that the Rab27b antibody is specific for Rab27b in acinar cells. In order to test for antibody specificity for Rab27b, mouse Rab27b primary antibody was pre-absorbed with His-tagged recombinant Rab27b protein overnight (~10 μg protein per 1 μg antibody) at 4°C, prior to immunostaining procedures in cultured LG acinar cells as described in **Experimental Procedures**. In the positive control, non-absorbed Rab27b (*green*) labels the subapical region beneath lumina delineated by abundant actin (*red*) labeling. This Rab27b signal is quenched with a pre-absorption step. A secondary only negative control is shown to indicate that the secondary for Rab27b does not show nonspecific background. Bars are 10 μm.

Supplement 2. Rab27a is detected at very low expression levels biochemically and by immunofluorescence. Using the same lysate samples used to detect Rab27b in **Figure 2**, a signal for Rab27a was probed by Western blotting (*c* is a control non-transduced LG acinar cell lysate, WT/CA/DN represent LG acinar cell lysate transduced with the respective constructs). Results are representative of three separate experiments. Rab27a showed no detectable endogenous signal but did show a low level of nonspecific recognition of the over-expressed YFP-tagged Rab27b (~ 10% of the signal obtained by the Rab27b antibody). Serial dilutions of recombinant His-tagged Rab27a protein showed that Rab27a antibody does recognize Rab27a binding sites, although endogenous Rab27a expression levels appeared too low to be detectable. Immunofluorescence for Rab27a conducted in parallel with Rab27b indicated similar conclusions. Bar is 5 μm.

Supplement Movie 3. Demonstration of luminal continuity with extracellular media. Live cell imaging of YFP-tagged WT Rab27b (*green*) expressed in LG acinar cells, with Texas Red

Dextran 10 kDa added to the media as a fluid phase tracer (*red*), shows that the lumen is open to the culture media. Little SV activity is seen at resting phase. With CCH stimulation, SV undergo membrane fusion events with the APM which briefly allows entry of the fluid phase tracer into the cavity of the newly vacated SV, before SV membrane cavities are gradually absorbed back into the APM.

Supplement Movie 4. Three-dimensional reconstruction of YFP-tagged WT Rab27b expression in live LG acinar cells showed redistribution of Rab27b-enriched SV. Cultured LG acinar cells transduced with an Ad construct for YFP tagged full-length Rab27b showed SV in the subapical region around the lumen (*). With the addition of CCH for 15 min, some SV traffic closer to the lumen while SV immediately beneath the lumen appeared to fuse with the APM, resulting in a net decrease of subapical SV. Bar is 5 μm.

Supplement Movie 5. Live cell imaging of YFP-tagged mutant Rab27b before and after CCH stimulation reveal differences in SV localization. Sample movies from YFP-tagged Rab27b expressing the WT, CA, or DN forms show acini for 10 min at resting phase followed by 15 min of CCH stimulation, with each movie each accelerated 90:1. In WT, while little movement is seen in resting cells, stimulated Rab27b-enriched SV undergo SV to SV fusion and also fusion with the APM. *Red arrows* emphasize several SV to APM fusion events, which are distinguishable from SV to SV or movements within different planes of the cells. In CA, some SV to SV fusion occurs even at resting phase. This activity and also fusion with the APM appears increased once these cells are stimulated. Finally, DN cells show cytoplasmic Rab27b expression that is not clearly recruited to SV membrane. * is lumen, bar is 5 μm.

Supplement Movie 6. Latrunculin effects Rab27b-enriched SV fusion with the APM. YFP-tagged WT Rab27b expressed in LG acinar cells showed changes during stimulated release of SV when cells were treated with latrunculin B, which effectively depolymerizes actin filaments. In non-treated LG acinar cells, SV immediately undergo homotypic fusion as well as docking and fusion with the APM, the latter of which are highlighted with *red arrows*. In LG acinar cells pre-treated with latrunculin B, SV appeared to retain homotypic fusion events but did not fuse with the APM. * is lumen; bar is 5 μm.

Supplement 7. DKO mouse tears show increased levels of IgG. Tears collected from C57 (N=3) and DKO (N=4) mice into 2 μl, glass capillary micropipettes before (basal) and after (stimulated) topical application of CCH to the exposed LG were individually run on Western blots and probed for IgG. IgG was detectable in the stimulated tears of DKO mice but not in those from C57 mice.